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ORIGINAL ARTICLE

# The effect of olive leaf extract in decreasing the expression of two pro-inflammatory cytokines in patients receiving chemotherapy for cancer. A randomized clinical trial

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**KEYWORDS**

Oral mucositis;
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Benzydamine hydrochloride (Benzydamine HCl);
Proinflammatory cytokine;
Tumor necrosis factor-α (TNF-α);
Interlukien-1 β (IL-1β)

**Abstract** *Background:* Oral mucositis is the most common side effects of chemotherapy of all cancer with intensive treatments regimen, and is the most common side effects of head and neck radiation therapy. For steam cell transplantation, its also regarded as the most debilitating side effects.

*Aims of the study:* The objectives of this study were to assess the effect of a mouth rinse containing olive leaf extract (OLE) in preventing severe oral mucositis in patients receiving chemotherapy, and to estimate its effect in decreasing pro-inflammatory cytokine production after chemotherapy.

*Materials and methods:* This study utilized a placebo-controlled, randomized, double-blind, and cross-over design. Twenty-five patients undergoing intensive chemotherapy were randomly assigned to receive a mouth wash containing OLE, benzydamine hydrochloride, or placebo in 3 different cycles of chemotherapy. Oral mucositis severity was assessed using the World Health Organization criteria and Oral Mycositis Assessment Scale. Patients were evaluated weekly until 15 days after chemotherapy for each cycle. Salivary levels of interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) were evaluated by enzyme-linked immunosorbant assay.

*Results:* Oral mucositis rates and severity after 2 weeks were significantly lower in the OLE and benzydamine groups compared to the placebo group. The IL-1β and TNF-α levels were significantly decreased in the OLE group compared to the other groups.

*Conclusion:* Preliminary findings indicate that OLE is effective in reducing IL-1β and TNF-α levels after chemotherapy and exert a therapeutic effect and prevent development of severe oral mucositis.



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## 1. Introduction

Oral mucositis is a common, debilitating, and painful side effect of chemo- and radio-therapies of head and neck malignancies (Elad et al., 2011). By virtue of their rapid mitotic rate,

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mucosal cells in the lining of gastrointestinal tract are natural targets of cancer cytotoxic regimens (Raber-Durlacher et al., 2010). At the clinical level, oral mucositis typically manifests as atrophy, swelling, erythema, and ulceration. The condition may be exacerbated by local factors, such as trauma from teeth, or microbial colonization (Raber-Durlacher et al., 2010). Oral mucositis has a significant impact on the patient's quality of life. Severe oral mucositis is one of the leading causes of unplanned treatment interruption, chemotherapeutic dose reductions, and changes in the selection of anti-neoplastic agents (Chen et al., 2011).

Recently, a biological model for chemotherapy- and radiotherapy-induced oral mucositis was proposed by Sonis et al. (2004), which revealed the complexity of the pathogenesis of this disease. The model described mucositis events in 5 overlapping phases: initiation, signaling with messenger generation, amplification, ulceration, and healing, with pro-inflammatory cytokines playing an important role. Cytokine release can lead to tissue injury, apoptosis, and loss of epithelial integrity, with consequent ulcer development. Due to the complex pathological process of oral mucositis, no intervention is available to prevent or treat the condition on its own. Many interventions can be found in the literature, including some that may be highly protective in one set of circumstances, but have little or no effect or may even be detrimental in others (Duncan and Grant, 2003). Researchers have suggested the need to combine interventions that act on different phases of mucositis (Rodriguez-Caballero et al., 2012).

Palifermin is among the recent target therapies for mucositis. This drug alters the cytokine profiles, specifically down-regulating tumor necrosis factor (TNF) (Potting et al., 2006; Logan et al., 2007). This finding has provided further support for the role of cytokines in the development of mucosal toxicity (Spielberger et al., 2004; Potting et al., 2006). Benzydamine hydrochloride, an oral rinse with analgesic, anesthetic, anti-inflammatory, and antimicrobial activities (Sonis, 2004; Silverman, 2007), has been shown to decrease the risk of oral mucositis development in several clinical settings (Kim et al., 1986; Prada and Chiesa, 1987; Epstein et al., 1989). It also appears to reduce erythema and ulceration after radiotherapy (Epstein et al., 2001) and chemotherapy (Cheng, 2004; Cheng et al., 2004).

Olive leaf extract (OLE) is a natural product that has been used as a medicament since ancient times. Throughout history, the olive plant has been an important source of nutrition and medicine. The therapeutic use of the olive plant has even been mentioned in Holy books. OLE exerts antioxidant (Visioli and Galli, 2002), anti-inflammatory (de la Puerta et al., 2000), and antimicrobial activities against bacteria (Walker, 1996), viruses (Lee-Huang et al., 2007), fungi, and mycoplasma (Aziz et al., 1998; Markin et al., 2003). Traditionally, OLE has been used to treat and prevent hypertension through its hypoglycemic, antiseptic, and diuretic properties (Coni et al., 2000; Manna et al., 2004; Andreadou et al., 2007; Singh et al., 2008). Recent studies have demonstrated the anticancer effects of OLE (Hamdi and Castellon, 2005; Abaza et al., 2007). For example, Atai et al. (2007) compared topical OLE with topical dexamethasone elixir for the treatment of recurrent aphthus ulceration, finding that both medications similarly reduced ulcer size and decreased pain. Only one study has examined the effect of OLE in reducing cancer-related complications; a doctoral dissertation study by Talabani et al. (2010) evaluated the effect of OLE in preventing and treating oral mucositis.

To our knowledge, no study in the literature has evaluated the effect of OLE on pro-inflammatory cytokine expression in cancer patients receiving chemotherapy. Accordingly, the purpose of this study was to investigate the ability of OLE to prevent or delay the appearance of severe oral mucositis in cancer patients receiving chemotherapy. Its effect was compared with that of benzydamine hydrochloride as a positive control and placebo as a negative control. Cytological assays were used to examine the effects of the drugs on the profiles of two pro-inflammatory cytokines: interleukin-1 beta (IL-1β) and TNF alpha (TNF-α).

## 2. Patients, materials, and methods

### 2.1. Setting and patients

The study was carried out at the Hiwa Oncology Hospital in Sulaimani City (Kurdistan region/Iraq) between December 2011 and June 2012. Twenty-five consecutive cancer patients (children and adults) who were under intensive cancer treatment participated in this study. All procedures were conducted in accordance with the guidelines approved by the local ethics committee of the University of Sulaimani. Fig. 1 shows a schematic for the design of the study.

The inclusion criteria of this study were as follows: (1) patients receiving intensive cancer treatment (high dose of a single cytotoxic drug or combinations of multiple cytotoxic drugs); (2) absence of prophylactic local treatment for mucositis; and (3) informed consent provided by the patient or their parents/guardians. A patient was excluded from participation in the study if any of the following exclusion criteria applied: (1) patients under non-intensive chemotherapeutic treatment; (2) patients taking prophylactic local medication for oral mucositis; and (3) patients who requested to leave or be excluded from the study.

### 2.2. Study design

A prospective, randomized, double-blind, placebo-controlled, cross-over study design was selected. There were a few reasons for the choice of study design. First, it was extremely difficult to control for all therapy- and patient-specific variables in a single-center study. Second, from a practical perspective, it was difficult to obtain sufficient numbers of participants in the study time period who met all of the inclusion criteria.

### 2.3. Oral treatment regimen

*Mouth rinse solutions*

- OLE (333 mg/ml *Olea europaea L.*, St. Francis Herb Farm Inc. Combermere, Canada)
- Benzydamine hydrochloride (0.15 g/100 ml, EPICO, Egypt; under License of F.ANGELINI ITALY®).
- Normal saline, as placebo.

Eligible patients were randomized to receive benzydamine hydrochloride, OLE, or placebo in the form of a mouth rinse. This oral treatment was changed in the next chemotherapy cycle for each patient (cross-over design). The studied drugs were self-administered 3–4 times daily for 14 days, starting on the

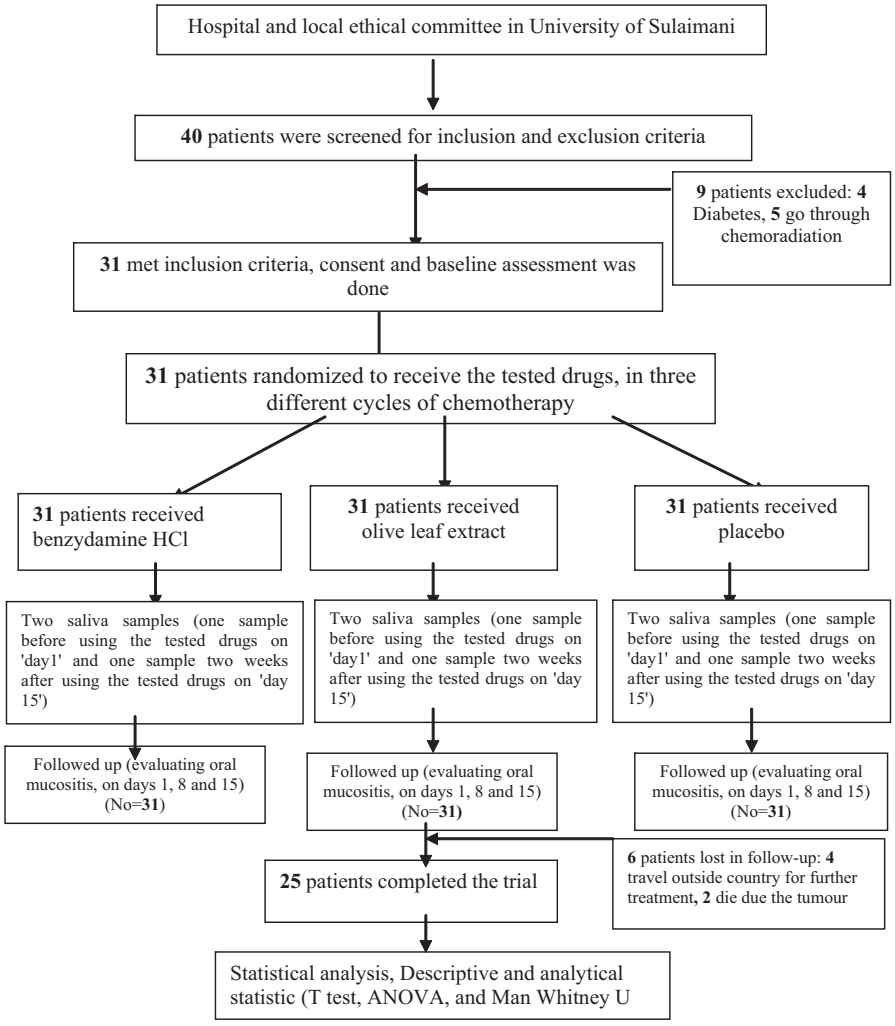


**Figure 1** Showing design of the trial.

first day of chemotherapy. Patients were asked to rinse their mouth with water before applying the oral treatment, to remove any remnants of food particles. They were also asked to maintain good oral hygiene by brushing with a soft bristle brush daily.

### *2.4. Scoring and monitoring oral mucositis*

During the study period, the tested oral treatments were visually evaluated on days 1, 8, and 15 of each cycle of chemotherapy. On these days, a follow-up case sheet was filled out for each patient.

Oral mucositis was scored according to the Oral Mucositis Assessment Scale (OMAS) and the World Health Organization (WHO) score as described by WHO (1979) and Sonis et al. (1999). Oral mucositis was measured before the mouth wash was used on days 1, 8, and 15. The two scoring systems were applied to be able to compare outcomes with findings from the literature that used either scoring system. The OMAS measures 9 sites in the mouth for erythema, pseudomembranes, or ulcerations. The mean score of OMAS ranges from 0 to 5. The WHO score is as follows: grade 0 = normal, no mucositis; grade 1 = soreness and erythema; grade 2 = erythema, ulceration, can eat solids; grade 3 = ulcers require liquid diet only; and grade 4 = alimentation not possible.

### *2.5. Collection of saliva samples*

Saliva samples were collected from each patient before starting the oral treatment (on day 1) and at the end of oral treatment (on day 15). Whole unstimulated saliva (WUS) was collected between 9:00 and 11:00 AM by the method described by Zhang et al. (2008). Patients were asked to refrain from eating and drinking for at least 30 min before sampling. All patients were requested to swallow first, tilt their head foreword, and then expectorate all saliva into a sterile container without swallowing for 5 min.

Samples were immediately transported to a laboratory and centrifuged for 20 min at 4000 rpm. If a cell pellet was not visible, then the sample was recentrifuged. The cleared saliva supernatant was decanted into a 1.0 ml sterile Eppendorf tube. All saliva samples were immediately frozen at −80 °C for further usage.

Following the manufacturer's instructions (Biotech, USA), assays for IL-1β and TNF-α were performed.

### 2.6. Statistical analysis

For all samples, data were collected in a case sheet, entered into the EXCEL software program, and analyzed using the Statistical Package for Social Sciences (SPSS) version 13 software package. Simple descriptive analysis was used for some variables (gender, age, and tumor type). Different statistical tests, such as analysis of variance (ANOVA), Spearman rank correlation coefficients, and Fisher's exact, Mann–Whitney, and chi-square tests, were used to find associations between different variables. A $P$-value $<0.05$ was regarded as statistically significant.

## 3. Results

### 3.1. Demographic and baseline characteristics

Table 1 lists the demographic data and tumor types for all patients. Most patients had leukemia (Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL)). The mean age of participants was 32 years, the age range was (10–40).

### 3.2. Oral mucositis assessment

The mean OMAS results on different days are shown in Tables 2 and 3. The lowest mean values were recorded in the OLE group, followed by the benzydamine and placebo groups, respectively. Changes in the OMAS results were statistically highly significant (P value $\leqslant 0.01$). The mean OMAS values were low on day 1 (first day after receiving chemotherapy). On days 8 and 15, the mean OMAS values were increased significantly in the placebo group compared to the OLE and benzydamine groups. Oral mucositis grades according to WHO grading system are shown in Table 4. The OLE group showed no grade 3 or 4 results. Grades 2, 3, and 4 were more common in the placebo group compared to the benzydamine group.

### 3.3. Level of pro-inflammatory cytokines in WUS

The IL-1β and TNF-α levels in the WUS of patients receiving chemotherapy were significantly decreased after applying OLE for 2 weeks. Levels of both cytokines, especially IL-1β, were significantly increased

**Table 1** Patient characterization, type of tumor and drug groups.

| Variables | Frequency (%) |
|---|---|
| *Sex* | |
| Male | 13(52%) |
| Female | 12(48%) |
| *Ages* | |
| Age range | (32.0) |
| Mean ± SD | 20.5 (9.0) |
| *Type tumor* | |
| AML | 7(28%) |
| ALL | 7(28%) |
| Burkitt Lymphoma | 6(24%) |
| HL | 2(8%) |
| NHL | 2(8%) |
| Multiple myeloma | 1(4%) |

AML: acute myeloid leukemia, ALL: acute lymphoblastic leukemia, HL: hodgkin's lymphoma, NHL: non hodgkin's lymphoma.

**Table 2** The mean OMAS mucositis score for the tested drugs at different times.

| Tested drugs | OMAS at different times (days) | | |
|---|---|---|---|
| | Mean ± SD | | |
| | 1 | 8 | 15 |
| | **$P < 0.05$** | **$P < 0.001$** | **$P < 0.001$** |
| Benzydamine HCl | 0.04 ± 0.12 | 0.43 ± 0.42 | 0.18 ± 0.22 |
| Olive leaf extract | 0.06 ± 0.14 | 0.11 ± 0.15 | 0.02 ± 0.04 |

*$P$ value statistically highly significant between OMAS and tested drugs, Benzydamine HCl: Benzydamine Hydrochloride.

**Table 3** Mean OMAS mucositis score for the tested drugs using *post hoc* test.

| Drugs | Times (days) | | |
|---|---|---|---|
| | Mean OMAS ± SD | | |
| | 1 | 8 | 15 |
| | **$P < 0.05$** | **$P < 0.001$** | **$P < 0.001$** |
| Benzydamine HCl | 0.04 ± 0.122 | 0.43 ± 0.429 | 0.18 ± 0.227 |
| Placebo | 0.16 ± 0.218 | 1.06 ± 0.293 | 0.60 ± 0.246 |
| | **$P > 0.05$** | **$P < 0.01$** | **$P < 0.01$** |
| Benzydamine HCl | 0.04 ± 0.12 | 0.43 ± 0.42 | 0.18 ± 0.22 |
| Olive leaf extract | 0.06 ± 0.14 | 0.11 ± 0.15 | 0.02 ± 0.04 |
| | **$P > 0.05$** | **$P < 0.001$** | **$P < 0.001$** |
| Olive leaf extract | 0.06 ± 0.14 | 0.11 ± 0.15 | 0.02 ± 0.04 |
| Placebo | 0.16 ± 0.21 | 1.06 ± 0.29 | 0.60 ± 0.24 |

*$P$ value statistically highly significant between OMAS and tested drugs.

**Table 4** The WHO grades for the tested drugs at different times.

| WHO Grading at different times | Tested drugs | | | $P$ value |
|---|---|---|---|---|
| | Benzydamine HCl N (%) | Olive Leaf N (%) | Placebo N (%) | |
| *Day 1* | | | | |
| Grade 0 | 22(88.0) | 20(80.0) | 13(52.0) | **$P < 0.05$** |
| Grade 1 | 2(8.0) | 4(16.0) | 11(44.0) | |
| Grade 2 | 1(4.0) | 1(4.0) | 1(4.0) | |
| *Days 8* | | | | |
| Grade 0 | 8(32.0) | 15(60.0) | 0(0.0) | **$P < 0.001$** |
| Grade 1 | 7(28.0) | 8(32.0) | 1(4.0) | |
| Grade 2 | 7(28.0) | 2(8.0) | 6(24.0) | |
| Grade 3 | 3(12.0) | 0(0.0) 0(0.0) | 15(60.0) | |
| Grade 4 | 0(0.0) | 15(60.0) | 3(12.0) | |
| *Days 15* | | | | |
| Grade 0 | 13(52.0) | 22(88.0) | 0(0.0) | **$P < 0.001$** |
| Grade 1 | 9(36.0) | 3(12.0) | 5(20.0) | |
| Grade 2 | 3(12.0) | 0(0.0) 0(0.0) | 15(60.0) | |
| Grade 3 | 0(0.0) | 22(88.0) | 5(20.0) | |

Benzydamine HCl: benzydamine hydrochloride, WHO: world health organization.

**Table 5** The level of TNF-α before and after using the tested drugs.

| Different materials | Tumor necrosis factor-α | | *P* value |
|---|---|---|---|
| | Pre treatment Mean ± SD | Post treatment Mean ± SD | |
| Benzydamine HCl | 48.4 ± 81.7 | 55.3 ± 1.2 | 0.817 |
| Olive leaf extract | 101.5 ± 64.5 | 51.9 ± 56.2 | **0.006** |
| Placebo | 76.3 ± 2.4 | 188.9 ± 3.2 | 0.172 |

The bold font shows the significant *P* value.

**Table 6** The level of IL-1β before and after using the tested drugs.

| Different materials | Interleukin 1-β | | *P* value |
|---|---|---|---|
| | Pre treatment Mean ± SD | Post treatment Mean ± SD | |
| Benzydamine HCl | 78.3 ± 76.2 | 66.6 ± 69.3 | 0.572 |
| Olive leaf extract | 117.7 ± 1.6 | 21.2 ± 53.3 | **0.001** |
| Placebo | 51.4 ± 55.5 | 112.4 ± 71.0 | **0.001** |

The bold font shows the significant *P* value.

after using placebo for 2 weeks. In the benzydamine group, the levels of both cytokines were decreased, but there were no significant changes (Tables 5 and 6).

## 4. Discussion

Despite the current understanding of the complex development of oral mucositis in cancer patients, no interventions are available for the prevention or treatment of this disorder. Interventions that target only one aspect of the mucositis pathobiological process have been reported to be largely ineffective (Stokman et al., 2006). Treatments should be directed toward multiple biological targets of the mucositis process, either by using an intervention with multiple mechanistic effects or using a combination of interventions. Palifermin has largely been accepted as the drug of choice (within certain limitations) for the prevention and treatment of mucositis (Spielberger et al., 2004; Blijlevens and Sonis, 2007; Sonis, 2007, 2009). However, this drug is given through an intravenous route, not as a topical application. Thus, cancer treatment centers continue to search for new drugs for oral mucositis prevention and treatment.

To conduct clinical trials of mucositis prevention, it is necessary to have reliable, valid, sensitive, and easy-to-use instruments. Through considerable effort, various mucositis scales have been developed for cancer patients undergoing chemotherapy and radiotherapy. The WHO scale is a functional and subjective scale for the clinical assessment of patients receiving cancer therapy, whereas the OMAS is a detailed objective scoring scale that was designed for clinical research trials. In the current study, both scales were used for clinical assessment of oral mucositis severity. The purpose behind using both scales was to assess the effect of the tested drugs on the subjective, functional, and objective outcomes of oral mucositis severity. According to Sung et al. (2007), the use of both instruments should provide a measure of both the severity and effects of mucositis (i.e., impact on the ability to eat and drink).

According to the pathobiology of oral mucositis (Sonis et al., 2004; Sonis, 2007), an increase in pro-inflammatory cytokines is associated with mucositis development and likely plays important roles in mediating injury and signaling. The intensity of pro-inflammatory cytokine production is increased before tissue damage and precedes the clinical appearance of oral mucositis (Yeoh et al., 2005; Sonis, 2007; Logan et al., 2007; Logan et al., 2009). This fact might explain the high levels of IL-1β and TNF-α in all 3 groups before administration of the tested treatments on the first day after chemotherapy. After the tested treatments were applied for 2 weeks, the intensities of both cytokines were decreased in the OLE and benzydamine groups, whereas the placebo group showed an increase in cytokine levels. This finding might be related to the effect of the tested treatments, with the placebo revealing lower activity toward the studied cytokines.

Although clinically the benzydamine and OLE groups showed lower mean OMAS and less severe WHO results compared to the placebo group, a statistically significant reduction in pro-inflammatory cytokine levels was only observed in the OLE group. A reduction in the expression of these cytokines is important for several reasons. First, the risk of oral mucositis remains and increases cumulatively with each cycle of chemotherapy. Second, the mucositis pathobiology (Sonis, 2007, 2009) suggests that submucosal damage in the endothelium will develop even in the absence of clinically visible lesions in the oral epithelium. In other words, our findings indicate that oral rinsing with OLE decreased the risk of developing severe oral mucositis for the next cycle of chemotherapy.

In conclusion, this study demonstrates, for what we believe to be the first time, that the use of a mouth wash containing OLE during chemotherapy can decrease the expression levels of IL-1β and TNF-α. This result might be due to the fact that OLE is effective against a wide range of oral microorganisms (Walker, 1996; Lee-Huang et al., 2007; Aziz et al., 1998; Markin et al., 2003). By reducing the impact of the oral microbial flora, OLE can help reduce cancer therapy-related complications and prevent further activation of pro-inflammatory cytokines through a negative feedback mechanism, as elaborated in the 5-stage model of oral mucositis (Sonis et al., 2004; Sonis, 2007, 2009).

Another explanation might be the antioxidant (Visioli and Galli, 2002) and anti-inflammatory (de la Puerta et al., 2000) nature of OLE. Benzydamine has also been demonstrated to exert antioxidant and anti-inflammatory effects, by decreasing the synthesis of IL-1β and TNF-α (Sironi et al., 1997; Ryu et al., 2007; Niscola et al., 2009). However, in the present study, benzydamine showed a weaker anti-inflammatory effect against IL-1β and TNF-α compared to OLE at 2 weeks after chemotherapy. This finding might suggest that OLE has a stronger anti-inflammatory action compared to benzydamine. Within the limitations of the study like the number of patients, the study period, and the randomization by computer block, these preliminary results indicate that further studies are warranted to investigate the effects of OLE in decreasing cancer-related complications.

## Conflict of interest

None declared.

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